3 With the Author's Compliments

OBSERVATIONS

ON THE

URINE IN PERNICIOUS ANÆMIA.

I. EXCRETION OF PATHOLOGICAL UROBILIN.
II. EXCRETION OF BLOOD PIGMENT.
III. EXCRETION OF IRON IN HEALTH AND DISEASE.

BY

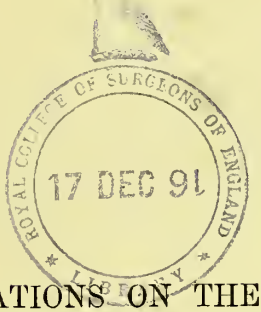
WILLIAM HUNTER, M.D., M.R.C.P., F.R.S.E.,

John Lucas Walker Student in Pathology, University of Cambridge; Arris and Gale Lecturer Royal College of Surgeons, England.

From the CAMBRIDGE PATHOLOGICAL LABORATORY.

PRINTED BY
RICHARD CLAY AND SONS, LIMITED,
BREAD STREET HILL.

Reprinted from THE PRACTITIONER.]



OBSERVATIONS ON THE URINE IN PERNICIOUS ANÆMIA.

BY WILLIAM HUNTER, M.D., M.R.C.P. (L. AND E.), F.R.S.E.,

John Lucas Walker Student in Pathology, University of Cambridge; Arris and Gale Lecturer, Royal College of Surgeons, England.

I.

EXCRETION OF PATHOLOGICAL UROBILIN.

I PROPOSE in this and following papers to record some observations I have recently made on the urine in a case of pernicious anæmia; and I do so the more willingly because they serve to supplement in some important particulars the observations I have already recorded regarding the symptomatology[1] and pathology[2] of this disease.

The full clinical details of the case, that of a man aged 58, I shall reserve for another occasion. At present I shall only refer to such features as were specially characteristic, and will at the same time serve to establish the diagnosis of the disease.

The observations on the urine were made during the last two and a half months of his illness, when I saw the patient from time to time along with Dr. Ingle. The weakness and debility, to which the patient succumbed on May 22 of the present year

[1] "Is Pernicious Anæmia a Special Disease?" *Practitioner*, vol. xli. p. 81 (Aug. 1888).

[2] "The Pathology of Pernicious Anæmia," *Lancet*, ii. 1888.

(1889), dated back to the end of 1887. During the autumn of that year the existence of this form of anæmia was first suspected. During the following winter he was able to carry on his literary work, although suffering a good deal from debility, and so far recovered that in the summer of 1888 he was able to carry out a tour he had planned, and even to indulge in a fair amount of physical exercise. From this he seemed to derive some benefit; but late in the same year the weakness again returned, and from that time (December 1888) onward he became gradually weaker, although from time to time there were short intervals during which he seemed to regain some of the lost ground.

The main feature of the case throughout was gradually increasing weakness without any apparent cause and without any corresponding degree of emaciation. In addition to this the chief symptoms were referable to the alimentary system. These consisted mainly of sore throat, dating back so far as November 1886, with soreness and tenderness of the mouth and tongue, dating back to November 1887. The condition of the tongue when first seen by me in June 1888 presented points of great interest. It was raw, extremely flabby, and indented at the margins, smooth on the surface, and devoid of epithelium. It presented also patches of more fiery redness over its surface and at its margins, the redness extending to the anterior pillars of the fauces. The condition of the tongue and fauces varied much from time to time; sometimes passing off for a time, but again returning, and causing much tenderness and pain when stimulating foods and drinks of any kind were taken. When at its worst, the patchy redness was frequently associated with the formation of small inflamed vesicles both on the anterior pillars of the fauces and under the root of the tongue. The pain was such that the patient described the sensation on swallowing as one of intense burning, extending the whole way down the throat into the stomach.

In the later stages of the illness this condition greatly improved; for the last six weeks he could take any kind of food or drink without the slightest discomfort.

Gastric symptoms were absent throughout. During the latter part of his illness a marked feature was the occurrence of

diarrhœa, or in its absence an irritation of the bowels with frequent and urgent calls to stool. These attacks generally occurred at night or in the early morning, and were frequently associated with profuse perspirations. For the last three or four weeks these symptoms also underwent amelioration: the perspirations were checked, and at the same time there was less disturbance of the bowels.

With regard to the other symptoms it may be noted, as of special importance in connexion with the condition of the urine presently to be described, that *fever* was not a feature of the illness. During the last two and a half months it was only present for short periods at a time, and never lasted longer than two to three days. Its occurrence was always connected with an increase of the general weakness and exacerbation of the other symptoms; but it was not in itself sufficient to cause the aggravation, for at no time did the temperature rise above 100° F., and more usually it was a little over 99° F.

Towards the last the patient presented a well-marked *lemon colour,* which varied from day to day in a very striking manner, and sometimes even at different times on the same day. At times the colour was with difficulty distinguishable from that of slight icterus; but only on one occasion did I note the condition of the conjunctivæ as one of slight jaundice. The situation where, curiously enough, I found this icteric tinge most marked was the mucous membrane of the soft and hard palate.

Epistaxis was a troublesome symptom in the earlier stages of the disease. During the last three months it only occurred twice, and was but slight. The rapidly increasing weakness towards the end was not caused by loss of blood, but occurred in periodic exacerbations, each attack leaving the patient still more reduced in strength.

The *changes in the blood* were marked throughout. At the time I first saw the patient (June 1888), the red corpuscles numbered 3,200,000 (64 per cent.) per cubic millimetre, with 56 per cent. of hæmoglobin. This high percentage of hæmoglobin, as compared with that observed in traumatic anæmia or in chlorosis, in which with the same number of corpuscles I have generally found the hæmoglobin as low as

20 per cent., was the point that satisfied me that the case was one of pernicious anæmia, although at the time the patient had recovered in a remarkable manner from an attack of great weakness during the previous winter: the number of corpuscles in February, as I am kindly informed in a letter from Dr. Lauder Brunton, having been so low as 860,000 per cubic millimetre (17 per cent.). Though complaining still of weakness, he was fairly well in health, and had resumed his literary work.

A month before death the number of corpuscles had fallen to 920,000 (19 per cent.), with 22 per cent. of hæmoglobin.

The relatively high percentage of hæmoglobin was thus maintained throughout. To this peculiar relation of the hæmoglobin percentage to the number of the corpuscles I attach a very high importance. I consider it *the only characteristic feature presented by the blood in pernicious anæmia.* It is never met with, according to my experience, in the anæmia resulting from loss of blood.

Poikilocytosis—changes in size and shape of the red corpuscles—was also marked.

The high degree of oligocythæmia and poikilocytosis so frequently met with is also common to traumatic anæmia provided it be of sufficient intensity, and is therefore only of diagnostic importance as regards the existence of pernicious anæmia when occurring in the absence of any previous loss of blood.

The *spleen* was recognisably enlarged during the last three months.

The diagnosis made during life was confirmed after death by an examination of the various organs. There was no trace of malignant disease. The liver was fatty, and presented on section a slightly rusty-brown mottled appearance, such as I have found to be almost characteristic in this disease. This appearance is due to fatty changes in the centre of the lobule, and the presence of much pigment in the more peripheral portions. A piece of the tissue placed in a solution of sulphide of ammonium became coal-black in the course of a few moments, a colour-reaction due to the presence of much iron in the form of pigment within the liver-cells. The diagnostic significance and importance of this test I have fully shown in my papers on the pathology of this disease (*loc. cit.*).

The few clinical and pathological details which I have deemed

GENERAL CHARACTERS OF THE URINE.

Date.	Quantity.	Reaction.	Specific Gravity.	Colour.
	ozs.			
March 9	35	Acid.	1015	Clear, and very high coloured.
,, 10	—	Ditto	1020	Ditto ditto
,, 11	38½	Ditto	1020	Ditto ditto
,, 12	18+	Ditto	1020	Ditto ditto
,, 13	18+	Ditto	1016	Ditto ditto No sediment.
,, 14	34	Ditto	1016	Slightly muddy; a heavy deposit of uric acid crystals. After filtration less high coloured than before; rosy-red deposit on filter.
,, 15	19+	Ditto	1014	Still muddy, but less so than yesterday; colour appreciably less than that of the 13th; a heavy deposit of uric acid crystals.
,, 18	21+	Ditto	1015	Urine clear, and of dark sherry colour. No deposit of urates or uric acid.
,, 19	22+	Ditto	1016	
,, 20	22+	Ditto	1017	Cloudy; heavy deposit of urates.
,, 21	20+	Ditto	1018	Very high coloured, but clear.
,, 22	37	Ditto	1014	Colour not so high.
,, **23**	36	Ditto	1016	*Colour very high;* urine clear. No deposit of urates or uric acid.
April 17	20+	Ditto	1010	Lighter in colour than at any time yet seen.
,, **18**	20+	Very acid.	1018	*Very high coloured;* deposit of uric acid.
,, 23	37	Acid.	1017	Still very high. Clear; no deposit.
,, 24	28+	Ditto	1016	Colour high; heavy deposit of uric acid on standing.
,, 25	37	Ditto	1016	Ditto ditto
,, 26	27+	Ditto	1015	Ditto ditto
,, 29	42	Ditto	1015	Colour slightly muddy.
,, 30	28+	Ditto	1014	Ditto ditto
May 1	42	Ditto	1016	Ditto very high.
,, 2	40	Ditto	1014	Ditto ditto
,, 3	49	Ditto	1010	Ditto ditto
,, 4	35	Ditto	1012	Ditto ditto
,, 6	35	Ditto	1013	Ditto ditto
,, 8	52	Ditto	1013	Ditto ditto
,, 9	42	Ditto	1014	Ditto ditto
,, 10	35	Ditto	1015	Ditto ditto
,, 11	49	Ditto	1014	Exceedingly high coloured.
,, 13	35	Ditto	1014	Ditto ditto
,, 14	52	Ditto	1016	Ditto ditto
,, 15	52	Ditto	1016	Ditto ditto
,, 16	42	Ditto	1014	Ditto ditto
,, 17	41	Ditto	1015	Ditto ditto
,, 18	52	Ditto	1015	Ditto ditto
,, 20	42	Ditto	1016	Ditto ditto

it necessary to give will, I trust, serve to show that the case was one of *pernicious* anæmia, and was not merely one presenting some points of resemblance to that disease.

The Urine:—Quantity. The quantity placed at my disposal from day to day during the last two and a half months of the patient's illness varied from 18 to 52 ounces. These figures represent only approximately the quantities daily passed, for in the earlier stages, as already noted, there was a good deal of intestinal disturbance, and much of the urine was therefore necessarily lost. During the last three weeks, when there was less disturbance of this nature, and the patient was in a condition of exceeding weakness and taking little nourishment, the quantity varied from 40 to 52 ounces.

The *Reaction* was acid throughout; sometimes very acid.

Specific Gravity. This varied from 1010 to 1020. The time over which the observations extended may be divided into three periods. During the first the average specific gravity was 1016; during the second 1015; and during the third 1014. The fall in the third period was doubtless due to the circumstance that the patient's diet at this time was mainly milk.

Colour. The colour of the urine was the most striking feature throughout. This was exceedingly high, varying slightly from time to time, sometimes even from day to day, but in all cases remaining very much higher than ever observed in conditions of health. As I only saw the case from time to time, in consultation with Dr. Ingle, I found myself able to some extent to judge of the condition of the patient from day to day by the colour of the urine sent to me. The colour was that of an extremely dark sherry, presenting generally at the same time something of an olive tinge. The urine was lightest in colour on April 17, and this change was associated with an improvement in the condition of the patient the most marked at any time observed. On this day there occurred a sudden and marked exacerbation of all his symptoms, with great weakness, drowsiness, marked lemon colour, dark colour of stools; and the urine again becoming very dark. The same relation between these attacks and the colour of the urine was observed on April 23. In neither instance did the specific gravity rise correspondingly, as usually occurs in fever. This absence of

relation between the colour of the urine and its quantity or specific gravity was best observed in the later stages of the illness, when, as I have stated, the quantity was greatest and the specific gravity lowest; and yet it was at this time that the colour was highest.

The urine was usually perfectly clear and transparent. On two or three occasions it was slightly muddy, and on two occasions (March 14th and 15th), at a time when arsenic was being pushed a little too rapidly, it was very muddy and threw down a heavy deposit of urates with uric acid crystals. At the same time there were some pain and difficulty in micturition, which soon however passed off.

With regard to the cause of this high colour of the urine, at no time were any *bile pigments* to be detected. In addition to the ordinary method of applying the Gmelin test, I used the one recommended by Hoppe-Seyler, when other colouring matters such as hæmoglobin or urobilin are present, namely precipitating the urine with a moderate quantity of milk of lime, shaking well, then filtering off the precipitate, and washing with water. A few drops of impure nitric acid allowed to fall on the precipitate on the filter gives the well-known colour reactions if any bile pigments are present.

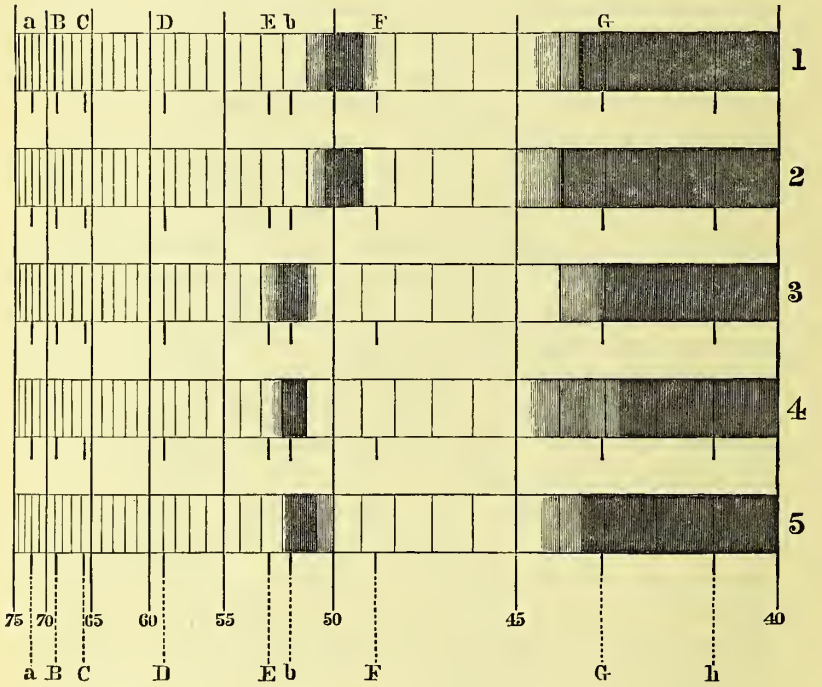
On the few occasions on which there was a heavy deposit of urates, there was a considerable quantity of *uro-crythrin*, the colouring matter so frequently found in the urine of fever patients, giving a well-marked rose-red colour to the paper when filtered. Hæmoglobin was never found either on chemical or spectroscopic examination.

On spectroscopic examination, however, I found a well-marked absorption band lying close to the line *F* of the spectrum, and fading off towards the line *b*, with at the same time a considerable absorption of the outer part of the blue spectrum (Figs. 1 and 2).

The colouring matter which gave this spectrum I was able to separate in large quantities by shaking the urine gently for some time with half its volume of chloroform, decanting off the solution, and then evaporating to dryness. After again being taken up in chloroform, filtered, and evaporated, a residue of a brownish-red colour remained, readily soluble in alcohol, the

solution showing the same well-defined chloroform band as in the urine itself.

On addition to the urine of a few drops of an alcoholic solution of chloride of zinc, a very marked green fluorescence was developed; the band became somewhat narrower, its darkest part being still on the side next *F*, and at the same time displaced more



Absorption-spectra of Urinary Pigment in Pernicious Anæmia.

1. Solution of pigment in alcohol.
2. The same more diluted.
3. Solution after addition of a few drops of zinc chloride.
6. The same diluted.
5. The same after further addition of a few drops of ammonia.

towards the line *b* (Figs. 3 and 4). On further addition of a few drops of ammonia the spectrum still showed an absorption band between *F* and *b*. Its characters however were changed, its darkest portion now being seen close to *b*, and the band fading off towards *F* (Fig. 5).

Both as regards its spectrum and its chemical behaviour

(green fluorescence with zinc chloride), the colouring matter present in such large quantities had all the characteristics of *pathological urobilin.* The spectrum is almost identical with that figured by Dr. MacMunn,[1] as that of pathological urobilin from a case of intraperitoneal hæmorrhage.

It is possible that in addition to the pathological urobilin there was also present in the above case some *urohæmatoporphyrin*, since, as MacMunn shows, the presence of a band at *F* in an acid solution, the development of a green fluorescence with zinc chloride and ammonia, and the shifting of the band towards the red with ammonia (Fig. 5) are as characteristic of urohæmatoporphyrin as of pathological urobilin. The fact however that the green fluorescence was so marked on the addition of zinc chloride alone, leads one to conclude that in the above case the colouring matter was mainly pathological urobilin.

The presence of this colouring matter in such quantities in pernicious anæmia has a twofold significance, both pathological and diagnostic.

That in all cases pathological urobilin is a product derived from the disintegration of hæmoglobin there is no doubt. As MacMunn has shown, pathological urobilin is quite distinct from the urobilin of healthy normal urine. However deep the layer of fluid may be, the absorption band at *F* of this normal urobilin never appears broad and black like that of pathological urobilin (Fig. 1); and, further, with the former no green fluorescence is developed with zinc chloride. This observation I consider to be one of great value and importance. It points to a difference in the conditions leading to the appearance of normal and pathological urobilin in the urine.

Both of them have in all probability a double origin—from hæmatin, and, by a process of reduction, from the pigments of the bile.

MacMunn's observations go far to show that normal urobilin is in all probability derived from hæmatin in the tissues, perhaps also from the hæmatin of the food. It is with difficulty obtained from bile-pigments, while it is easily obtained from hæmatin.

On the other hand pathological urobilin is in all probability

[1] "On the Origin of Normal and Pathological Urobilin," *Journal of Physiology*, x. p. 71, plates x. and xi.

derived mainly from the bile-pigments, and to a less extent from hæmatin. In the bile MacMunn has indeed found a urobilin-like substance, convertible by oxidation into one closely resembling pathological urobilin.

Whether derived therefore from the bile-pigments, or from this urobilin-like substance excreted with the bile, we have at least ample evidence, in the above case, that the excretion of pathological urobilin was connected with a largely increased secretion of bile. A darker character of the fæces was noted from time to time in association with the recurrent aggravations of weakness. At the same time the urine contained much more colouring matter. In one case of pernicious anæmia I found still more certain evidence of this increased secretion of bile. The duodenum and upper part of the small intestine contained a very large quantity of highly concentrated bile, extremely rich in pigments.

I think there can be no doubt therefore that in the excretion of such large quantities of pathological urobilin we have extremely valuable evidence as to the essential nature of this disease, namely, that it depends, as I have maintained elsewhere, on an excessive destruction of blood:—in other words, that pernicious anæmia is hæmolytic in its nature.

In the present case the richness of the urine in colouring matters could have had absolutely no relation to the absorption of colouring matters derived from the food, for during the latter part of the illness the diet on which I had recommended the patient to be placed was as little nitrogenous as possible, and consisted mainly of milk.

In this connexion it may be pointed out, as bearing on the derivation of this colouring matter from the blood indirectly through the bile, that the cases hitherto described in which pathological urobilin has been found in greatest quantity have been instances in which large extravasations of blood were in process of absorption. A largely increased formation of bile pigments follows the destruction of the hæmoglobin supplied to the liver in increased quantity under such circumstances.

In addition to its origin from the pigments contained in the bile, I conceive it is likely, however, that another probable source of the pathological urobilin in pernicious anæmia, is the pigment

found, as my former observations show, in such great abundance within the liver. I am the more inclined to arrive at the conclusion from the appearances presented *post mortem* in the foregoing case. The urine remained of high colour to the very last, but on *post mortem* examination I found no excess of bile in the duodenum or small intestine. On the contrary, the mucous membrane was covered merely with slightly bile-stained mucus. The probable origin of pathological urobilin in other organs of the body from products of blood-destruction, as well as from the pigments of the bile within the intestinal tract, is thus to be kept in mind.

Further, in addition to increased formation of bile pigments, there was in all probability a second factor concerned in the appearance of such large quantities of pigment in the urine in the above case, namely, increased absorption from the intestinal tract. The evidence pointing to this conclusion I hope to consider in the following papers, where I shall deal with the excretion of iron and of aromatic compounds in the above case.

Finally, as regards its bearing on the diagnosis of the disease, the excretion of such large quantities of colouring matter in the urine, entirely independent, be it noted, of the occurrence of fever or of any diminution in the quantity of the urine, or rise in the specific gravity, is, I think, of the greatest interest and importance.

In my former observations my conclusions regarding the hæmolytic nature of this disease were based solely on (1) a consideration of the anatomical changes to be found after death, and (2) on the possibility of inducing experimentally similar changes in animals by the action of blood-destroying agents. It was obvious that evidence of this destruction would in all probability be found in the urine, but in the absence for several years of any opportunity of seeing a case of this disease, and of making any observations with regard to this point, I considered it better to defer the consideration of this evidence.

Dr. Bristowe, and more recently Dr. Mott in an extremely interesting paper, have also directed attention to the high colour of the urine in cases of pernicious anæmia coming under their notice. In the light of the foregoing observations it will become more and more a matter of importance to observe the condi-

tion of the urine in all cases of anæmia of doubtful nature. The only anæmia liable to be mistaken for pernicious anæmia, both as regards its general features and the degree of change in the blood, is the anæmia resulting from loss of blood. I have already indicated that as regards the changes in the blood in the two forms, important differences exist which enable one to state with some degree of certainty whether the anæmia is *pernicious* or *traumatic*, the chief one being the relatively high hæmoglobin percentage in the former.

It now becomes clear that a far more important difference must exist as regards the condition of the urine in the two forms. The urine of traumatic anæmia is invariably extremely pale, and I have found this the case even when some degree of fever is present.

The pathological processes underlying pernicious anæmia must, on the other hand, always be associated with a formation of effete pigments and colouring matters, evidence of which ought invariably to be found. Such evidence in the above case I judge was found—

(1) In the marked lemon-colour presented by the patient, which varied, as I have stated, in a remarkable manner, the variations corresponding with a higher colour of the urine.

(2) In the slight degree of jaundice observed at one time, and so frequently met with in other cases.

(3) In the increased formation of bile-pigments as shown by the dark colour of the fæces.

(4) In the high colour of the urine.

If, in addition, the colouring matter present be found to be pathological urobilin, I should consider this fact (in the absence of fever, or of any diminution in the quantity of the urine, the latter at the same time being of low specific gravity) as absolutely diagnostic of the existence of pernicious anæmia.

II. Excretion of Blood-pigment.

Having in my former paper discussed the significance of the pathological urobilin excreted in large quantities in the case described, I propose now to consider certain other characters presented by the urine, which seem to me of some interest and importance.

In my study of the pathology of pernicious anæmia [1] I was led to the conclusion, from a consideration of the anatomical changes I found in the kidneys, that microscopic examination of the urine might be found in certain cases to throw not a little light on the nature of the anæmia we had to deal with. This conclusion I find fully borne out by the results of my observations in the present case.

To make the observations I am now about to record the more easily understood, it will be necessary for me to make what may appear a slight digression, and to recall very briefly some of the more important facts regarding the pathology of pernicious anæmia established by my former observations.

The chief result of these observations was to show (1) that the essential feature of this form of anæmia is excessive destruction, and not in the first instance any impaired formation, of blood; and (2) that the characteristic feature of this blood-destruction, by which it is in the first place to be distinguished from that occurring in paroxysmal hæmoglobinuria, is that it is initiated in and for the most part limited to the portal circulation, the chief seats of the destruction within this area being the spleen, the capillaries of the liver, and the radicles of the portal system within the gastro-intestinal walls.

This limitation of the destructive process serves to explain why the liver is so constantly the seat of the important and characteristic changes then described, inasmuch as all the hæmoglobin set free is necessarily carried to that organ in the first instance. It also serves to explain why hæmoglobinuria is not a symptom of the disease, notwithstanding that in many cases a considerable and sometimes very sudden destruction of

[1] *Practitioner*, xli. 81, and *Lancet*, ii. 1888.

blood takes place. The hæmoglobin liberated by such an occurrence is within certain limits entirely disposed of while passing though the liver, either by being excreted in the form of bile-pigments or by being stored up in the form of blood-pigments.

I concluded, however, that the blood-destruction is not always confined within these limits. The course of the disease is usually marked by exacerbations more or less periodic, during which the amount of blood-destruction may be so great that the liver is unable to dispose of all the hæmoglobin supplied to it. The hæmoglobin then passes into the general circulation, and is excreted, as all analogy would lead us to infer, by the kidneys. Evidence of such an excretion I found in the large quantity of pigment present in the kidneys in certain cases of the disease I had examined, the situation and character of which alike pointed to the hæmoglobin of the blood as its source.

That under such circumstances hæmoglobin or any of its immediate derivatives had never been described as present in the urine was a remarkable fact, and seemed at first sight to negative the view that hæmoglobin in any form was excreted by the kidneys.

In the case now under consideration I had an opportunity of satisfying myself as to the freedom of the urine from such evidences of blood-destruction. During the last three months of the patient's illness there were two well-marked exacerbations such as I have referred to, both of them sudden in their onset and attended by great weakness, drowsiness, slightly increased temperature (not exceeding, however, 100° F.), a more marked lemon tint of the skin, and an exceedingly high colour of the urine.

Certain of these clinical phenomena, more especially the excretion of the large quantities of pathological urobilin already described, undoubtedly pointed to an increased destruction of blood, comparatively sudden too in its occurrence. Nevertheless, at no time was hæmoglobin or any of its immediate derivatives, such as methæmoglobin or acid hæmatin, to be found in the urine.

The discrepancy thus manifest between the clinical phenomena and the pathological events I have supposed to occur at this time is, however, more apparent than real.

As I have endeavoured to explain in my study of the pathology of the disease, the absence of hæmoglobin or any of its immediate derivatives from the urine in such cases is to be explained by the peculiar *form* in which the hæmoglobin reaches the kidneys, and the *channel* by which it is excreted. Instead of being free, the hæmoglobin liberated from the corpuscles remains combined in some peculiar way with the albuminous constituents of the corpuscle or of the plasma.

The evidence pointing to this conclusion I have considered elsewhere,[1] and I will therefore not dwell upon this point further than to add that urine containing hæmoglobin in this form does not show any of the reactions of the hæmoglobin. When it appears in the urine, it does so in form of small yellow "albuminous droplets," only recognisable on microscopic examination.

I find moreover that there is a marked difference between the changes to be found in the kidneys in pernicious anæmia and those found in ordinary hæmoglobinuria. While in the latter the chief changes to be found are the presence of *menisci* of hæmoglobin in and around the glomeruli, and sometimes similar albuminous casts in the tubules, in pernicious anæmia the changes are limited to the renal epithelium, and consist in the presence of fine granular blood-pigment within the epithelium of the convoluted tubules, none being found in the glomeruli.

This difference in the character of the changes in these two conditions points I believe to a difference in the channels of excretion of the colouring matter in the two cases.

In ordinary hæmoglobinuria the principal, most observers consider the only, channel by which the hæmoglobin passes into the urine is through the glomeruli. If any excretion at all takes place through the epithelium of the tubules, this is altogether of secondary importance to that which occurs through the glomeruli. On this point my observations leave me no room for doubt, and in this conclusion I am at one with all other observers. Hence it is that the chief evidences of this excretion are to be found in and around the glomeruli.

In pernicious anæmia, on the other hand, the absence of

[1] *Lancet*, ii. 1888, p. 657.

pigment from the glomeruli, and its presence in the epithelium of the convoluted tubules, must in my opinion be regarded as pointing to the epithelium as the chief channel of excretion of the hæmoglobin.

An analogy pointing to a similar difference in the channels of excretion is afforded in the case of salts of iron or indigo-carmine after injection into the blood. Glœvecke [1] found that after injection of salts of iron into the blood, their excretion takes place through the epithelium of the convoluted tubules, and not through the glomeruli; and the preponderance of evidence goes to show [2] that the same holds true for indigo-carmine when similarly injected, the granules being found in process of excretion within the renal cells.

The idea naturally suggests itself in this connexion that in pernicious anæmia we have really to do with an excretion of some remote derivative of hæmoglobin, containing all the iron of the original hæmoglobin molecule. And this idea cannot be altogether set aside as unfounded, although the balance of evidence is, so far as my observations at present go, in favour of the view already stated—that it is not merely an iron-derivative of the hæmoglobin, but a modified form of hæmoglobin itself, which passes into the renal cells and is excreted.

Description of Changes in the Kidney in Pernicious Anæmia.

The foregoing conclusions as to the channel by which hæmoglobin is excreted in pernicious anæmia are based on a study of the changes presented by the kidneys in well-marked cases of the disease. The nature of these changes I have represented in the accompanying wood-cut.

For the material of the case from which the above was taken I am indebted to the kindness of Dr. Byrom Bramwell.[3] I append here a description of the changes found in the above

[1] *Maly's Jahresber ü. Thier-Chemie*, 1883, p. 182.

[2] See Foster, *Text Book of Physiology*, 5th ed. vol. ii. p. 667.

[3] I gladly seize this opportunity of acknowledging my indebtedness to Dr. Byrom Bramwell for his kindness in placing at my disposal all his available pathological material. To this circumstance I owe much of the opportunity I have had for the study of the morbid anatomy of the disease.

case, and would merely add that the description given may be taken as applying to all the other cases (twelve in number) I have so far had the opportunity of examining, in which pigment has been present. In none of the cases, however, has the pigment been so abundant, or the changes so marked.

On placing sections first in a solution of ferrocyanide of potassium and afterwards in dilute hydrochloric acid, a well-marked blue reaction is developed in the cortex, most marked close to its periphery.

On microscopic examination this change is found to be due to the presence of pigment lying within certain of the tubules, and giving the characteristic reaction of iron in free form. The pigment is in the form of fine granules, the individual granules being of spherical shape and fairly uniform size, and their diameter varying from 1 to 2 microns (μ).

The distribution of the pigment amongst the tubules is peculiar. It seems to be confined to the convoluted tubules, the deeper blue coloration of the cortex being due to the presence of pigment in the tubules in this region. More careful examination shows that this is the case : all the pigment is to be found within the convoluted tubules, and is fairly equally distributed between their primary and secondary convolutions.

A slight trace of pigment is also seen at certain portions of Henle's loops, those portions, namely, whose epithelium most closely resembles in character that of the convoluted tubules. With this exception the loops of Henle are free from pigment granules. Their epithelium presents at most a faintly bluish and diffuse coloration. The same faintly bluish tint is to be observed in the epithelium of the angular tubules.

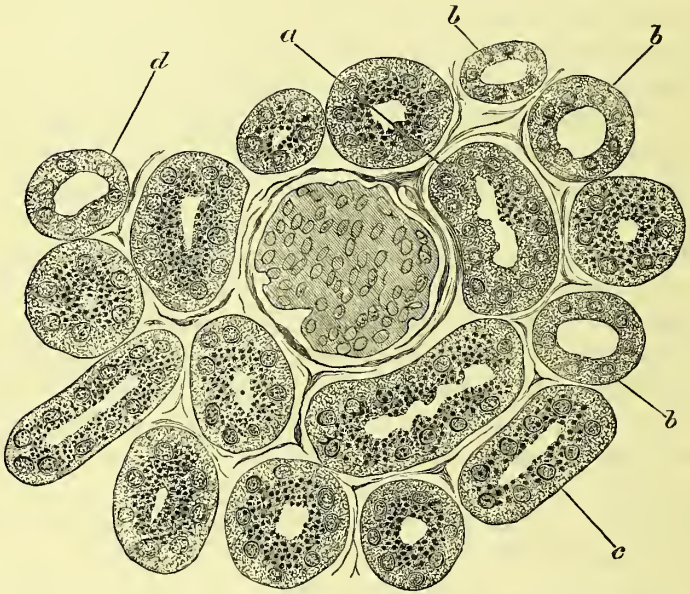
In the collecting tubes, not a trace of pigment of any kind, whether in diffuse or in granular form, is to be seen, and the same holds true of the glomeruli.

It is to be noted that all the convoluted tubules do not contain an equal amount of pigment. The amount varies much; while some are free, others contain very varying quantities. In all cases alike, the pigment lies within the cells of the tubules, not free within the lumen. In some of the convoluted tubules the pigment appears to fill up the lumen, an appearance seen on closer examination to be due to the circumstance that the

pigment lies close to the unattached free border of the cells (see figure).

With the exception of the presence of this pigment, the kidney shows nothing abnormal. The cells, even those containing the pigment, show no signs of degeneration, their nuclei staining readily.

I conclude from the appearances presented that a colouring



Section of the Kidney in Pernicious Anæmia : Blood-pigment in process of Excretion through the Epithelium of the Convoluted Tubules.

a, Normal convoluted tubule, one cell showing traces of pigment ; *b*, ascending limb of Henle's loop ; *c*, convoluted tubule, pigment lying within the epithelium ; *d*, descending limb of Henle's loop.

matter from the blood is in process of excretion through the epithelium of the convoluted tubules, and that while in process of excretion it has assumed a granular form. Within the cells the hæmoglobin has undergone disintegration, and become converted into blood-pigment. The bluish tint of the epithelium in certain parts of Henle's loops, indicative as it is of the presence of iron, I judge to be due rather to the absorption of some iron-containing constituent of the urine

passing along the tubules, than to any excretion of iron by them. A similar blue appearance I have found presented by the kidney epithelium in ordinary hæmoglobinuria.

Excretion of Blood-pigment in the Urine.

The absence of any trace of iron or of pigment from the glomeruli can only be explained by assuming that the excretion has taken place not through the glomeruli, but through the epithelium of the convoluted tubules.

Corresponding to the differences I have described, *first*, in the form of the colouring matter excreted in the two conditions of simple hæmoglobinuria and pernicious anæmia; *secondly*, in the evidences of its excretion to be found in the kidneys; and *thirdly*, in the channels by which it is excreted, there are I find equally important differences in the clinical evidence of such an excretion afforded by the urine.

To these I would now call attention. Evidence of the excessive blood-destruction characteristic of pernicious anæmia was to be found in the urine, in the case on which these observations were made, not only (1) in the presence of pathological urobilin as we have already seen, but also (2) in the presence during the exacerbations of renal cells and casts containing granules of blood-pigment, and (3) an increased excretion of iron.

March 9.—At the time I first saw the patient the most striking character presented by the urine was its high colour. On microscopic examination nothing abnormal was detected.

March 10.—A number of faintly granular cast-like structures are to be seen, holding yellowish pigment-granules which resemble blood-pigment very closely in appearance, size, and colour. The casts are short and fragmentary, and made up of partially degenerated cells apparently of renal origin, the outlines of the individual cells being indistinct. The yellowish pigment granules are confined to these cells, none being seen free. The granules are sufficiently large to show distinct outlines with the power used (× 250). Cells from the bladder are also seen, but they are free from any such pigment.

March 11.—Similar cells are seen arranged as before in cast-

like groups of three, four, or more, and containing yellow pigment-granules.

March 12.—A few isolated cells are seen containing the same yellowish granules. The pigment is not so abundant as before, and the cells, which are small and round and appear to be renal in their origin, are not so numerous. They are no longer arranged in groups.

March 13—18.—Daily examination of the urine has failed to reveal any cells such as were before seen. The urine on several days threw down a heavy deposit of urates and uric acid crystals. Its colour has in the meantime improved.

March 19—22.—Colour of the urine has again become higher, the specific gravity however still remaining 1015 to 1018. On the afternoon of *March* 22 the patient had a marked exacerbation of all his former symptoms—profound weakness with drowsiness, increase of lemon tint with apparently some slight degree of icterus of the conjunctivæ, looseness of bowels, and darker colour of stools.

March 23.—The urine markedly changed for the worse. Although the specific gravity is not increased (1016), the urine presents a very high colour; it is perfectly clear however, and throws down no deposit on standing. On microscopic examination, a number of degenerated renal cells are seen, partly single, partly arranged in cast-like groups of three, four, or more, most of them containing yellow pigment granules. These granules present exactly the appearance of blood-pigment, and are so numerous that some of the cells seem filled with them. No granules are to be seen free; they are all contained within renal cells or their remains. The appearance of these cells contrasts markedly with that of the few cells from the bladder also seen, the latter being perfectly free from this pigment. The urine is singularly free from amorphous urates, so that the appearance presented by the yellow pigment granules is all the more striking. The more these cells are examined the stronger becomes the conviction that they are renal in their origin, and that the pigment they contain is blood-pigment. The uniform size of the pigment-granules, their spherical shape, and yellow or brownish-yellow colour, are precisely the characters of blood-pigment granules.

I did not see the patient after this for a period of three weeks. In the meantime he had recovered from his attack of increased weakness, and had indeed gained ground. I found him on my return considerably improved in health, his appetite better, the colour returning to his lips and gums, and he himself feeling altogether stronger and better.

April 17.—Urine lighter in colour and more nearly normal than at any time yet observed. On the afternoon of this day there was a second exacerbation in all respects similar to the first.

April 18.—Colour of the urine again very high. On microscopic examination renal cells seen as before containing yellowish pigment granules, easily distinguishable by their colour and appearance from the surrounding urates. Some of the cells are grouped together in cast-like form, but most of them are single.

I need not give the further details of these examinations during the remainder of the illness. Cells, similar in character to those already described and with similar contents, continued to appear from time to time in the urine. For the patient's condition from the time of this last exacerbation became rapidly worse, the colour of the urine remaining continuously and persistently high, if anything, higher than before, notwithstanding that the quantity was undiminished and the specific gravity lower (1014).

What was the meaning of these changes found on microscopical examination? The consideration already given to the histological changes met with in the kidney supplies us, I think, with the answer to this question.

I was at first unwilling to accept the view which at once naturally suggested itself, namely, that the pigment found in such small quantity came from the kidney. The gradual diminution in the number of cells and in the quantity of pigment they contained from *March* 10 onwards, and the final disappearance of both cells and pigment after *March* 13, led me, however, to suspect that such might nevertheless be the case. For this disappearance was connected with a change in the patient's diet which I had suggested, and with what appeared to be a resulting slight improvement in his general condition.

The diet of the patient at the time I first saw him was of a highly stimulating and nitrogenous character, consisting of meat juices, beef-tea, &c. Having satisfied myself from my observations on the nature of blood-destruction [1] that such a diet was perhaps the best fitted of any to ensure an increased destruction of blood even in health, I felt desirous of substituting a blander and less nitrogenous one; and the suggestion I made to this end was accordingly adopted on *March* 11, two days after I first saw him.

The disappearance of pigment-cells from the urine, and the slight improvement in the health of the patient, seemed at first to justify this step. But the change for the better was only of short duration. Exacerbations more or less acute occurred at intervals, apparently uninfluenced by the treatment adopted. On this, however, I do not desire at present to dwell, as it may more fittingly be considered at another time.

The important point, so far as our present object is concerned, was that with each exacerbation there was a reappearance of these pigment-carrying renal cells in the urine. The connexion, in point of time, between the two occurrences was so close as finally to satisfy me that they stood to each other in the relation of cause and effect: the greater blood-destruction initiated by the exacerbation was, in fact, evidenced by the excretion of blood-pigment in the urine. The cells were such as had probably become detached from the convoluted tubules under the extra strain thrown upon them in the excretion of the pigmentary material supplied to them from the blood. The character of the pigment they contained was precisely such as I have already described as being found within the kidney.

It is perhaps unnecessary for me to dwell any further on the pathological bearing of this excretion of pigment, or its importance from a diagnostic point of view. The observations recorded speak for themselves. As regards its significance, the appearance of this pigment in the urine, in the form I have described, bears out fully what I have already said regarding the excretion of hæmoglobin by the kidneys in cases of pernicious anæmia, and the channels by which this excretion takes place.

[1] "Arris and Gale Lectures," *Brit. Med. Journ.*, ii. 1889.

As regards its bearing on diagnosis, the discovery of pigment cells in the urine in a doubtful case of anæmia, would, if combined with a high colour of the urine, and especially with an excretion of pathological urobilin, undoubtedly to my mind serve to establish the case as one of pernicious anæmia.

NOTE.—Since the above was written I have been able to examine microscopically the kidneys from the case I have described. The appearances found fully justified the diagnosis made during life. The convoluted tubules showed a large quantity of pigment in process of excretion, the form and distribution of the pigment being precisely similar to that described in the text. In many of the tubules the epithelial cells were filled with pigment-granules. A number of these showed signs of fatty degeneration. Some of the pigment was contained within cells that seemed to have become detached, and lay free within the lumen of the tubule.

III. The Excretion of Iron in the Urine in Health and Disease.

In the light of the observations already recorded regarding the excretion of hæmoglobin, and its appearance in the urine in the form of blood-pigment, it became a matter of some interest to determine what was the excretion of iron in pernicious anæmia as compared with that which occurs in health.

My former observations showed me that as the result of the excessive blood-destruction prevailing, there was an accumulation of iron in certain organs of the body—notably within the liver, and occasionally also within the kidneys. It seemed not improbable, therefore, that the urine might show some evidence of this greater destruction in the form of an increased excretion of iron. At the time my observations were made I was naturally unaware of the condition of the kidneys in the particular case described, that is to say, of the presence of a very large excess of pigment lying within the cells of the convoluted tubules. This condition obviously rendered a considerably increased excretion of iron all the more likely.

Iron is a constant constituent of the urine in health. It is present, however, in exceedingly small quantity, and in such a form as only to be recognisable on very careful analysis of the ash. The presence of free iron in the urine can be recognised, as Hamburger [1] has shown, by its characteristic reaction with sulphide of ammonium, even when in such dilution as 1 to 10,000. The iron normally present in the urine gives no reaction with this reagent. We must conclude, therefore, that it is present in organic combination with other constituents of the urine.

Whether this combination is similar to the one we are acquainted with in the case of hæmatin, or is of the same nature as in the nuclein combinations of the yolk of egg and in milk, we are as yet ignorant. In all probability it is of the former kind, and has its origin therefore in the hæmoglobin of the blood.

[1] Hamburger, *Zeitschrift für physiol. Chemie*, ii. 191 (1878).

Methods of Estimation.

The methods we at present possess for the estimation of the iron in the urine are unfortunately very imperfect; a matter of the more consequence, as the quantities we have to deal with are so exceedingly small as to render the errors that arise of the greatest possible importance. Iron is so abundantly present in nature, and is so frequently present in the various reagents used in its detection, that it is almost impossible to rid ourselves of this ever-present source of fallacy. Nearly all the observations hitherto made suffer in accuracy from fallacies arising in this way.

The methods hitherto in use have been chiefly two, that by weighing, and that by titration with a standard solution of potassium permanganate.

By Weighing.—The urine after evaporation to dryness in a platinum capsule is reduced to charcoal; and then carefully extracted with a moderate quantity of warm water, and filtered to remove the soluble salts (Filtrate No. 1). The insoluble residue left on the filter, along with the filter itself, is then completely ignited, till only a white ash remains. This is extracted first with dilute and afterwards with strong hydrochloric acid, and again filtered (Filtrate No. 2). The filtrate thus obtained contains, according to Hoppe-Seyler, the whole of the iron. So far is this, however, from being the case, that I find that a trace, although a small trace, of the iron occasionally passes through in the first filtrate, especially if a considerable quantity of water be used. It is desirable, therefore, to evaporate the first filtrate to dryness, extract with a small quantity of hydrochloric acid, filter again, and add the filtrate thus obtained to Filtrate No. 2.

The two filtrates thus combined contain the whole of the iron. On adding ammonia, a white flocculent precipitate is thrown down, made up of the phosphates of magnesium and calcium, along with phosphate of iron, to which form in the process of ignition the iron of the urine is reduced.

The phosphates of magnesium and calcium are readily soluble, with the aid of heat, in acetic acid, in which reagent the phosphate of iron is insoluble. The separation of the latter can there-

fore be effected by carefully filtering through a small filter of known ash-composition; and after being carefully dried and ignited, its quantity can be determined by weighing. The precipitate thus obtained contains the iron in the form of a ferric phosphate (Fe_3PO_4); and since this latter contains 37·09 per cent. of iron the quantity of iron present in it may be determined.

According to Hoppe-Seyler, this method gives results of sufficient accuracy in the case of fluids, such as the urine, that contain phosphoric acid in large excess; the iron present is then converted into a phosphate. It was the one I employed in my earlier estimations. It is one, however, on the accuracy of whose results we cannot by any means constantly rely. The results obtained give in most cases too high an estimate. It is difficult in any case to be certain that the precipitate finally obtained is made up entirely of phosphate of iron, although by carefully warming with acetic acid this can in many cases be accomplished.

It need scarcely be said that in all these operations every care must be taken to avoid introduction of iron from dust in the air, through the reagents employed, or through the filter paper. In the case of the filter paper it is necessary to use one of definitely known ash-composition. The weight thus determined is subtracted from the total weight of the precipitate after final ignition.

By Titration.—In all cases it is better and safer to estimate the amount of iron by reducing it to the form of a ferrous salt, and then determining its reducing power on a solution of potassium permanganate of known strength. For this purpose the iron is converted into ferric sulphate, and then reduced to a ferrous state by means of zinc. Titration with the permanganate solution is then carried out in the usual way.

Certain sources of fallacy are met with in using this method also. There are not infrequently impurities in the zinc, and these may have a reducing action on the permanganate solution, even when no ferrous salt is present. The error arising in this way has been regarded by one observer (Jacobi) as constant, being equivalent to not less than 1·8 milligramme of pure iron per litre. When it is remembered that, as will presently be

seen, the whole of the iron in the urine frequently amounts to little more than 1 or 2 milligrammes, it will be understood how grave a source of fallacy this may prove to be.

Another method has recently been proposed and carried out by Gottlieb.[1] This is based on an entirely different principle from the above, namely the conversion of the iron into Prussian blue and its determination by weighing. The method is complicated and requires the use of a large number of reagents, in itself a source of grave error.

In Gottlieb's hands, however, it seems to have yielded remarkably constant results, and if his observations are confirmed the method may prove a valuable addition to those we already possess. It has the advantage of being specially suitable for determining very small quantities of iron.

Excretion of Iron in Health.

Owing mainly to the imperfections in our methods, partly also to the slow and tedious nature of the investigation, the observations hitherto made with regard to the daily normal excretion of iron in the urine have been but few.

Magnier[2] found in a healthy man the excretion to vary from about 4 to about 16 milligrammes per day, a wide range of variation, equivalent to an average daily excretion of about 10 milligrammes.

Hamburger found traces of iron constantly present in the ash of the urine, both in health and disease. In two healthy women he found a daily excretion of 10·1 and 15·6 milligrammes respectively, giving an average for the two of a little over 12 milligrammes. There cannot, I think, be a doubt that this estimate is too high. The method Hamburger used was that of titration, the iron being reduced to a ferrous state by warming with sulphurous acid instead of zinc. I find that in this method it is impossible afterwards to get rid of all traces of the sulphurous acid, and this exercises a very strong reducing power on the per-

[1] Gottlieb, *Archiv f. experim. Pathol. u. Pharmakol.*, November 1889.

[2] Magnier, *Maly's Jahresber. ü. Thier-Chemie*, 1875, p. 138.

manganate solution, in many cases even exceeding that of the ferrous salt actually present.

The results of Gottlieb's observations give a much lower estimate. In five cases he found it to vary from 3·69 to 1·59 milligrammes, giving an average of 2·59 milligrammes per day.

The excretion from day to day in each individual case remained remarkably constant, the widest range of variation in any one case being 0·21 milligramme, and the average range of variation from day to day in the whole series being only 0·15 milligramme.

These results differ widely from those obtained in the fewer and more limited observations of Magnier and Hamburger. If they are to be relied on, and the narrow range of variation found is their best recommendation, they indicate a daily excretion of iron considerably less than that of 10 milligrammes hitherto accepted.

From my own observations (four in number) presently to be recorded, I conclude that the average daily excretion may most probably be taken as ranging from 3 to 5 milligrammes. In a healthy individual I found the daily excretion to be 5·65 milligrammes. After allowing for the error of excess which usually attends the method of estimation employed in this instance, that by weighing, this result agrees with the above estimate. In three cases of chlorosis, in two of which the patients were getting large doses of iron, the daily excretion found was 1·71, 1·96, and 1·61 milligrammes respectively, giving an average of 1·76 milligrammes. The average in the two conditions was thus 3·70 milligrammes, any error of excess in the first being counterbalanced by the slight diminution in excretion which probably existed in the second.

Influence of Various Conditions on the Excretion of Iron.

This appears to be remarkably slight. The excretion seems to remain fairly constant for each individual.

It is not affected, at least directly, by changes in the quality or quantity of food. Thus Hamburger found, in a dog, that the

daily excretion remained the same whether the animal was receiving 300 or 500 grammes of meat daily. What is more remarkable, it is apparently not affected by the administration of even large quantities of iron by the mouth.

The observations with regard to this point divide themselves naturally, both in point of time and in point of result, into three series.

The view long held, that iron administered by the mouth was absorbed as such, and utilised for purposes of blood-formation, had to be abandoned when Hamburger (1878) showed that the absorption of iron in such cases was infinitesimal. In one experiment, out of 441 milligrammes of iron administered in the form of ferrous sulphate by the mouth, an excess of only 12 milligrammes was to be found in the urine over the period of time during which the administration lasted. That even this increase, slight as it was, was indirectly brought about, and was dependent on increased metabolism in the tissues rather than on increased absorption of the iron, was shown by the fact that in no case, however great the amount of iron given by the mouth, could free iron be detected in the urine.

Following on these observations came those of Kobert[1] (1883) and Cahn[2] (1884). Hamburger's observations had pointed to a slightly increased excretion as the immediate result of the administration of iron by the mouth. Kobert and Cahn found that under such circumstances no absorption at all took place from the intestine, and hence any increased excretion of iron if present must be solely an indirect result.

Lastly, differing still more from those obtained by Hamburger are the results obtained by Gottlieb. So far from being increased, his observations would almost appear to show that the excretion is diminished while iron is being administered by the mouth. The immediate effect of the administration was to diminish for the first two or three days the quantity excreted in the urine. The excretion rose again in the following days, but never above that at which it previously stood. Thus in a healthy

[1] Kobert, *Mangan und Eisen*, *Archiv f. exper. Pathol. u. Pharmak.* xvi. (1883).

[2] Cahn, *Resorptions und Ausscheidungs-verhältnisse des Mangans im Organismus*, *ibid.* xviii. (1884).

man, in whom the daily excretion of iron for three days had averaged 3·68 milligrammes, the excretion while iron was given by the mouth fell to 1·19 and 0·70 milligrammes, and on the third day the iron disappeared altogether. After the administration of the iron was stopped, the excretion rose again to 0·56 and then to 2·54 milligrammes.

In two other cases the same result was observed. It is extremely difficult to explain how such a result is produced, —nor need we stop to discuss it. The chief interest of these observations lies in this, that they seem conclusively to show that the excretion of iron in the urine is as little affected by iron administered by the mouth as it is by diet. This conclusion is one of some importance in endeavouring to determine what significance should be attached to any increased excretion in disease.

Excretion of Iron in Disease.

This subject has as yet received scarcely any attention. The only observations hitherto made are those of Hamburger already incidentally noted, in which he found traces of iron constantly present in the ash of urine in a large number of diseases, including, amongst others, jaundice, pneumonia, diabetes, typhoid fever, puerperal fever, pleurisy, chlorosis, and leucocythæmia. No attempt, however, was apparently made to estimate the quantities present in these diseases.

Some of Gottlieb's observations were made on patients suffering from nervous diseases, the excretion of urine in whom, as he took for granted, was a normal one. I find, however, from a study of his results, that while in an undoubtedly healthy individual the average excretion was 3·68, in a case of general paralysis it was 2·62, and in a case of "motor aphasia" it was 1·58 milligrammes. It is possible that Gottlieb may have been in error in regarding these cases as normal; and since he uses these results in arriving at his conclusion as to the average excretion in health, it is probable that this may account for the lowness of his estimate, namely 2·59 milligrammes.

My own observations number seven in all, one made in

health, three in chlorosis, and three in pernicious anæmia. The results are represented in the following table :—

No. of Observation.	Condition.	Quantity of Urine in ccm.	Specific Gravity.	Excretion of Iron in milligrammes.	Remarks.
1	Health.	1,500	1,018	5·65	
2	Chlorosis.	900	1,018	1·71	Has been receiving 6 grs. reduced iron daily for three weeks. Greatly improved.
3	,,	1,175	1,014	1·96	Has been receiving 30 ♏ Tr. Ferri Perchlor. daily for three weeks. Improvement great, although not so marked as in No. 2.
4	,,	1,365	1,015	1 61	Treatment not commenced.
5	Pernicious Anæmia.	1,100	1,014	32·26	*May* 2. (Death on *May* 22.) Urine of very high colour, contains much pathological urobilin. Not examined microscopically, but on *May* 1 contained a considerable number of renal cells, showing blood-pigment.
6	,,	1,500	1,014	6·52	*May* 14.—Patient profoundly weak. Colour of urine as high as before.
7	,,	1,200	1,016	1·00	*May* 20.—Patient in a moribund condition. Urine still of high colour.

The result of the observation made in health calls for little remark. It is considerably higher than the average obtained by Gottlieb (2·59), although at the same time considerably lower than the average obtained by Magnier and Hamburger (10 milligrammes).

The observations in chlorosis are not sufficiently numerous to enable one to speak with certainty as to the excretion of iron in this condition. So far as they go, they point to a diminished excretion, the average of the three observations being 1·76 milligramme, considerably lower, therefore, than the average obtained by Gottlieb in health, and still lower than the average estimated by myself. There need, I think, be the less hesitation in accepting this result, when the condition of the blood in chlorosis as regards richness in hæmoglobin is borne in mind.

Assuming what is most probable, namely that the iron excreted in the urine is derived directly or indirectly from the

hæmoglobin of the blood, one would on *a priori* grounds alone expect a diminished excretion where the blood is so poor in hæmoglobin as it is in chlorosis.

Observations Nos. 2 and 3 are also of interest as showing how little the excretion is influenced, directly at least, by the administration of iron by the mouth. Both patients had been receiving iron in considerable doses for three weeks previously, and both had improved under the treatment, without any apparent influence on the excretion of iron. This remained almost the same as that found in No. 4 where no iron had been given. The improvement was most marked and most rapid in No. 2; and the smaller excretion of iron in this, namely 1·71 milligramme, as compared with that in No. 3, namely 1·96, was in all probability related to the circumstance that the attack was the third, in a comparatively short period of time, from which the patient had suffered. At the time of admission she presented in a very marked degree the symptoms of extreme chlorosis.

The most striking feature of these observations, however and the one to which I would specially direct attention, is the enormous excretion found in one instance in the case of pernicious anæmia. My observations on the excretion of iron in this case were unfortunately not made till near the fatal termination of the case, when the patient was already in a state of profound weakness. That, notwithstanding his condition in this respect, the urine showed such a large excretion of iron, must undoubtedly be ascribed to the excessive blood-destruction constantly going on, as evidenced more especially by the high colour of the urine associated with the presence of so much pathological urobilin,[1] and the presence from time to time of considerable quantities of blood-pigment.[2] That under the circumstances, therefore, there should be such an increased excretion of iron as was found on *May* 2 need occasion but little surprise.

A very small amount of blood-pigment in the urine would amply account for an increase of this or even a still larger amount. The urine on this particular day was not examined microscopically,

[1] *Practitioner*, September 1889.
[2] *Ibid.* November 1889.

but on the day before I found a considerable number of renal cells in the urine, most of them containing pigment in considerable amount. It is very probable, therefore, that a similar condition existed on *May* 2.

How far an increased excretion of iron in pernicious anæmia is a constant condition, or how far, on the other hand, it is intermittent, occurring from time to time associated with the periodic exacerbations of the destructive process, it will remain for future observations to determine.

The results obtained in the present case point to the latter conclusion. Another estimation was made on *May* 14, a week before death, when the patient was in a condition of exceeding weakness, with occasional wandering and mild delirium. The excretion found (6·52 milligrammes) must be regarded as, relatively speaking, a very large one, considering the condition of the patient's blood at the time. So poor was the blood in hæmoglobin that it scarcely sufficed to give a red tint to a white cloth, as was observed on one occasion when there was a slight attack of epistaxis. The excretion on *May* 20, two days before death, had fallen still lower. This relatively large excretion, observed on *May* 14, I am inclined to regard as of equal significance to the much larger excretion observed on *May* 2. Both alike pointed to the conclusion that there was a largely increased destruction of blood, the excess of iron being derived from the blood-pigment, probably also from other iron-containing products of this destruction present in the urine.

Summary.

In bringing for the present this further series of observations on the pathology of pernicious anæmia to a close, it may be desirable, in conclusion, to summarise briefly the results arrived at in this paper and the two preceding ones.

In the urine of the case I have described there were three changes made out sufficiently well to merit some degree of attention.

(1) *The presence of pathological urobilin* in great quantity—a colouring matter derived in all probability partly from the bile-pigments in the intestinal tract, partly from other products

of hæmoglobin destruction, found so abundantly in the liver, spleen, and kidneys in this particular case.

(2) *The presence of blood-pigment*, recognisable on microscopic examination, of whose source there could be no doubt, as it appeared in the urine in the form of granules, similar in appearance and in character to those afterwards found in the cells of certain of the renal tubules.

(3) *An increased excretion of iron.*

All these changes point to the existence of one condition of the blood, namely an excessive destruction; and they all agree in one respect, namely that they varied in degree from time to time, the aggravations of weakness from which the patient periodically suffered being marked by higher colour of the urine, excretion of blood-pigment, and increased excretion of iron.

In addition to this, their pathological significance, these changes are, I am inclined to think, of no little importance from a diagnostic point of view. The high colour of the urine observed, unaccompanied as it was by any diminution in quantity or any rise in specific gravity, and the presence of granules of blood-pigment in the urine, pointed so unmistakably to the nature of the pathological process at work in the blood, that they established conclusively the diagnosis of the case as one of pernicious anæmia.

One must, however, in this connexion guard one's self against a misconception that may not improbably arise. The urine in pernicious anæmia need not *always* show these well-marked and, when present, characteristic changes. It may be said however, with some degree of assurance, that they will be found more or less marked in all cases at some period or other of their history.

In all cases, as in the foregoing one, there will be times corresponding to the periods when the patient is gaining ground, when the colour of the urine will be that of health, and nothing abnormal will be microscopically recognisable.

The aggravations of weakness will always, however, be evidenced by a higher colour of the urine, it may be also by the appearance of blood-pigment granules in the urine; both changes marking the nature of the process within the blood which is the occasion of these attacks, namely excessive hæmolysis.

In the recognition of this fact, as to the nature of this form of anæmia, and of the further fact that, as I have endeavoured to show elsewhere, this destruction is initiated in the portal system, and depends upon changes occurring within the gastro-intestinal tract, is to be found the great indication for that treatment of the disease which will most probably be of value. Having established, as I think these various observations conclusively do, what is the nature of the morbid process within the blood, our next endeavour must be to recognise and combat the changes within the gastro-intestinal tract on which they depend. This subject I hope to deal with in a future paper, based on further investigations either already made or still in progress.